Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1963, **29**, 711-720

International Standards for Anti-Poliovirus Sera Types 1, 2 and 3

J. LYNG [1] & M. WEIS BENTZON [2]

Anti-poliovirus sera types 1, 2 and 3 were examined in an international collaborative study to test their suitability as international standards. It was concluded that the sera are suitable for the purpose, and they are accordingly established as International Standards for Anti-Poliovirus Sera, Types 1, 2 and 3, replacing the existing International Reference Preparations for the same sera, established in 1958.

The unitage was defined as follows :

For Type 1 preparation : 1 unit is 10.78 mg.
For Type 2 preparation : 1 unit is 10.46 mg.
For Type 3 preparation : 1 unit is 10.48 mg.

INTRODUCTION

At its meeting in 1958 the WHO Expert Committee on Biological Standardization (1959) established International Reference Preparations of Anti-poliomyelitis Sera. At the same meeting, however, the Committee agreed, on the basis of a report suggesting that the neutralizing potency of the sera might vary from ampoule to ampoule, that these sera should, in due course, be replaced by International Standards and asked the Statens Seruminstitut, Copenhagen, to obtain suitable material for this purpose.

In 1959 the sera established as British standard poliomyelitis antisera, Types 1, 2 and 3 (Perkins & Evans, 1959) were offered from the National Institute for Medical Research, London, in sufficient quantity to be established as International Standards. This offer was accepted, and in July 1959, 500 ampoules of each type were received in Copenhagen.

This report describes an international collaborative assay with the object of determining the suitability of these sera to serve as International Standards for replacement of the above-mentioned International Reference Preparations.

At its meeting in September 1960, the WHO Expert Committee on Biological Standardization (1961) authorized the Statens Seruminstitut to establish these materials as the International Standards for Anti-poliovirus Sera of Types 1, 2 and 3 and to define the international units with the agreement of the participants in the collaborative assay.

Organization of assay (assay plan)

The participating laboratories received three sets of six or seven unknown sera, one set for each of the virus types. The sera were to be titrated on four different days on four different tissue-culture lots simultaneously with the proposed standard sera.

Participants were expected to use the method in current practice in the laboratory.

Participating laboratories

The following took part in the study:

H. v. Magnus, M.D., Statens Seruminstitut, Copenhagen, Denmark

R. Murray, M.D., National Institutes of Health, Bethesda, Md., USA

Professor R. Sohier, Section of Virology, National Public Health Laboratory, Faculty of Medicine, Lyons, France

Professor V. D. Soloviev, Moscow Institute for Poliomyelitis Prophylactics, Moscow, USSR

In this report the laboratories are designated by code numbers.

MATERIALS AND METHODS

Sera

Sera T_{1-3}, U_{1-3} and V_{1-3} were trivalent sera obtained by immunization with poliomyelitis vaccine. Although each serum is trivalent, serum T_1 was used

[1] Pharmacist, Department of Biological Standardization, Statens Seruminstitut, Copenhagen.

[2] Actuary, Bio-Statistical Department, Statens Seruminstitut, Copenhagen.

TABLE 1
DESCRIPTION OF METHODS

	Laboratory number			
	1	2	3	4
Combining time and temperature for virus-serum mixture	3½ hr/37°C for cytopathic test; 3hr/22°C for metabolic inhibition test	1 hr/20°C	1 hr/20°C	—
Factor by which serum was diluted between successive mixtures	2	2	2	2
Number of tissue-culture tubes (or cups) used per serum dilution	3 for standard, 2 for test sera	2	2	—
Virus strains employed:				
Type 1	Mahoney	Brunhilde	Mahoney	Brunhilde
Type 2	MEF-1	MEF-1	MEF-1	MEF-1
Type 3	Saukett	Saukett	Saukett	Saukett
Reading (microscopic or by colour change) and time of reading	Microscopic, 7 days; colour, 6 days	Microscopic, 7 days	Microscopic, 7 days	

only for titration with Type 1, serum T_2 only for Type 2, and so on for sera U and V as well.

Sera X_{1-3} were monospecific hyperimmune sera diluted in normal monkey serum. X_1 is monospecific for Type 1, X_2 for Type 2, and X_3 for Type 3.

Sera Y_{1-3} were 1 : 5 dilutions in normal monkey serum of sera X_{1-3}, respectively.

Sera Z_{1-3} were normal monkey sera and are identical for all three types.

Sera S_{1-3} were monospecific sera prepared by immunization with live virus suspensions prepared in monkey-kidney-cell cultures. The virus strains used were Mahoney (Type 1), MEF-1 (Type 2) and Saukett (Type 3). For each type the high-titre serum was diluted in 6% aqueous dextran solution to give a titre with respect to its neutralizing antibody of the order of 500-1000. The diluted sera were distributed in 1-ml volumes in ampoules and freeze-dried. These sera are identical with the sera established as British standard poliomyelitis antisera Types 1, 2 and 3 (Perkins & Evans, 1959) and are the proposed International Standards.

Sera R_{1-3} were the International Reference Preparations of Anti-poliomyelitis Sera (Bentzon et al., 1962).

Sera L_{1-3} were local sera.

Sera R and S were lyophilized. All other sera were in the liquid state and were held at +4°C.

The proposed international standard sera were prepared by the National Institute for Medical Research, London.

All sera were prepared from rhesus monkeys.

Methods

All laboratories used modifications of the cytopathic neutralization test (Gard et al., 1956). Laboratory 1 used besides a metabolic inhibition test (Salk et al., 1954).

Details of methods used in the different laboratories are given in Table 1.

Titres

Where the basic readings were available the titres have been evaluated by the modified Kärber method (Spaun, 1956). This is the case for laboratories 2 and 3.

The modified Kärber method is essentially a method for determination of the mid-point between the last dilution showing full reaction (two positives) and the first showing no reaction (two negatives). When no dilution giving full reaction has been tested, it is always assumed that the next lower dilution

step would have given two positives, and the sign < is applied. The computational procedure will be clear from the following five examples:

Serum dilution	*Log reciprocal dilution*	*Ex. 1*	*Ex. 2*	*Ex. 3*	*Ex. 4*	*Ex. 5*
1/16	1.20	—	—	—	—	—
1/32	1.51	2	2	1	1	0
1/64	1.81	2	1	1	0	0
1/128	2.11	2	0	0	0	0
1/256	2.41	0	0	0	0	0
Estimated log titre .		2.26	1.81	<1.66	<1.51	<1.35
Estimated titre . . .		180	64	<46	<32	<22

RESULTS

Basic results (titres)

In Table 2 are given the titres for each individual titration together with the challenge doses of virus in units of LD_{50}. Geometric mean titres per serum and laboratory are also shown.

The data from laboratory 4 are shown in this table but are not included in the further evaluation, as the many partial values would have complicated the calculations unduly. The excluded data seem not to contradict the general conclusions of the investigations.

Relative potencies

Each of the three standards was assigned an arbitrary potency of 10 units per ampoule. For each laboratory the relative potencies of the other sera were calculated in terms of the appropriate standard (by dividing the geometric mean titre of each serum by the geometric mean titre of the standard and multiplying by 10) and were expressed in units per millilitre. The relative potencies are given in Table 3, and it is seen from the table that the agreement between laboratories is reasonably good.

By comparing Tables 2 and 3 it will be noted that whereas in the cytopathic test the titres from laboratories 1 and 2 were generally three to four times higher than the titres from laboratory 3, differences of such magnitude were not found in the relative potencies. *It is thus demonstrated that for the cytopathic test with the use of a standard serum the results of polio serum titrations in different laboratories are directly comparable.*

In Table 2, the original titre values found for serum Y have been multiplied by 5 to obtain figures comparable to those for serum X, since serum Y was prepared by diluting serum X five times. The potency of serum Y thus adjusted was found to agree well with the potency of serum X in all laboratories.

A comparison between the results obtained in laboratory 1 in the metabolic inhibition test and in the cytopathic test reveals differences in the relative potencies estimated by the two methods. These differences for types 2 and 3 were greater than the over-all limits of error (for both methods) for that particular laboratory.

As only the standard serum and sera X and Y were hyperimmune sera, an avidity difference between these sera and the other test sera might be expected. But although certain differences in variation for the standard and for the other sera could be demonstrated, these could hardly have been due to avidity differences, as no correlation was found between the standard serum and sera X and Y in this respect.

The International Reference Preparations (sera $R_{1\text{-}3}$)

As the homogeneity of the International Reference Preparations (sera $R_{1\text{-}3}$), as mentioned, has been seriously questioned, a careful additional study was performed by Dr F. T. Perkins, Division of Immunological Products Control, National Institute of Medical Research, London. Ampoules stored in London as well as in Copenhagen were examined. The two sets of results were in good agreement. Average relative potencies of serum R are given in Table 4.

It is noted, however, that these results do not agree with the results given in Table 2. This disagreement remains unexplained.

Variances

In Table 5 the results of the analyses of variances (logarithmic) are tabulated. s_R^2 is the residual variance (the titration variance per laboratory), whereas s_T^2 is the variance between tests per laboratory. The mean titration variance for each laboratory is also shown. From these values standard deviation and standard errors have been calculated (Table 6).

The variances obtained in the cytopathic test in the three laboratories are not significantly different.

Weight variation per ampoule and stability

Tests for homogeneity between ampoules of the proposed standards had been performed before they

TABLE 2

POLIOMYELITIS ANTIBODY TITRES OBTAINED

A. Virus Type 1

Laboratory No.	Test	Titres obtained [a]									Virus challenge dose (× LD_{50})
		T_1	U_1	V_1	X_1	$Y_1 \times 5$	Z_1	S_1	R_1	L_1	
1	Cytopathic	64	48	96	512	320	<4	160	ND	ND	155
		96	32	64	512	320	<4	240	ND	ND	140
		128	48	128	512	320	<2	240	ND	ND	252
		256	96	128	1 536	640	<2	450	ND	ND	162
	Geometric mean	120	51	100	676	355	<3	251	ND	ND	174
	Metabolic inhibition	96	48	48	1 024	960	<4	240	ND	ND	226
		48	64	48	512	640	<2	240	ND	ND	236
		128	64	64	768	640	<2	320	ND	ND	300
		128	96	48	768	640	<2	480	ND	ND	176
	Geometric mean	93	66	51	759	705	<2	309	ND	ND	229
2	Cytopathic	91	64	91	363	910	<3	182	8	ND	63
		91	46	64	724	640	<1	182	8	ND	79
		91	91	32	724	455	<1	128	6	ND	32
	Geometric mean	91	64	58	575	640	<2	162	7	ND	55
3	Cytopathic	46	<16	16	512	320	<6	91	22	724	204
		64	46	22	256	230	<6	91	22	724	96
		64	22	<16	182	230	<6	91	46	724	40
		91	22	22	256	230	<6	91	22	1 024	83
	Geometric mean	64	25	<19	282	250	<6	91	27	789	89
4	Cytopathic	<64	64	<64	64	<320	<64	<64	<64	>64	—
		64	256	<64	<64	<320	<64	128	<64	256	—
		64	<64	64	64	320	<64	128	64	256	—
		128	64	128	256	320	<64	256	64	512	—
	Geometric mean	<78	<92	<78	<92	<320	<64	<128	<64	>219	—

[a] ND = not done.

TABLE 2 *(continued)*

POLIOMYELITIS ANTIBODY TITRES OBTAINED

B. Virus Type 2

Laboratory No.	Test	Titres obtained [a]									Virus challenge dose (× LD_{50})
		T_2	U_2	V_2	X_2	$Y_2 \times 5$	Z_2	S_2	R_2	L_2	
1	Cytopathic	192	48	24	512	640	<4	160	ND	ND	183
		192	96	32	384	640	<4	120	ND	ND	438
		128	48	32	512	480	<2	160	ND	ND	162
		384	128	48	1 024	960	<2	480	ND	ND	68
	Geometric mean	204	72	33	562	660	<3	195	ND	ND	174
	Metabolic inhibition	192	192	64	768	1 280	4	560	ND	ND	57
		128	192	48	768	960	2	480	ND	ND	100
		380	256	128	768	960	2	640	ND	ND	300
		380	256	128	1 024	1 280	2	640	ND	ND	185
	Geometric mean	246	224	85	832	1 120	2	575	ND	ND	135
2	Cytopathic	182	64	32	363	1 280	3	182	6	ND	40
		64	64	<8	363	320	1	91	11	ND	63
		91	128	11	512	910	1	128	22	ND	20
	Geometric mean	102	81	<14	407	725	2	128	11	ND	37
3	Cytopathic	91	<22	<11	363	230	6	<64	46	512	195
		46	22	<11	182	110	6	<64	46	724	58
		91	32	11	182	320	6	64	32	724	59
		64	46	<11	256	320	6	128	64	724	59
	Geometric mean	71	<30	<11	234	230	6	<78	46	664	79
4	Cytopathic	<64	<64	64	128	<320	64	<64	<64	128	—
		64	<64	64	128	<320	64	64	<64	512	—
		32	<64	<64	64	160	64	32	32	512	—
		64	<64	<64	64	320	64	<64	64	512	—
	Geometric mean	<55	<64	<64	91	<275	64	<55	<55	363	—

[a] ND = not done.

TABLE 2 *(concluded)*

POLIOMYELITIS ANTIBODY TITRES OBTAINED

C. Virus Type 3

Laboratory No.	Test	Titres obtained [a]									Virus challenge dose ($\times LD_{50}$)
		T_3	U_3	V_3	X_3	$Y_3 \times 5$	Z_3	S_3	R_3	L_3	
1	Cytopathic	256	96	16	1 024	1 280	<4	80	ND	ND	240
		384	128	16	768	640	<4	120	ND	ND	112
		128	64	32	768	1 280	<2	160	ND	ND	252
		256	128	12	1 024	2 560	<2	240	ND	ND	87
	Geometric mean	240	100	18	891	1 280	<3	138	ND	ND	155
	Metabolic inhibition	384	192	96	3 072	3 840	<4	480	ND	ND	100
		256	192	48	3 072	2 560	<2	560	ND	ND	176
		512	192	64	4 096	2 560	<2	640	ND	ND	300
		384	192	64	4 096	1 920	<2	640	ND	ND	156
	Geometric mean	372	192	66	3 548	2 625	<2	575	ND	ND	170
2	Cytopathic	256	91	<22	>1 466	1 815	<3	256	<6	ND	50
		182	128	<11	512	910	<1	182	8	ND	63
		512	128	16	2 048	2 560	<1	256	6	ND	32
	Geometric mean	288	115	<16	>1 150	1 620	<2	229	<6	ND	47
3	Cytopathic	64	46	<16	1 024	455	<6	46	22	1 448	129
		182	91	<11	363	455	<6	91	22	724	135
		128	46	<11	512	455	<6	128	16	1 024	47
		64	64	22	512	910	<6	91	22	2 048	20
	Geometric mean	100	59	<14	562	550	<6	83	20	1 217	63
4	Cytopathic	<64	<64	<64	<64	320	<64	64	64	ND	—
		512	128	<64	<64	1 280	<64	256	128	256	—
		64	64	<64	64	320	<64	32	32	128	—
		128	<64	<64	64	320	<64	64	64	256	—
	Geometric mean	<128	<78	<64	<64	455	<64	78	64	204	—

[a] ND = not done.

TABLE 3
MEAN RELATIVE POTENCIES OF SERA [a]

Virus type	Laboratory No.	Test	Relative potencies of sera (units per ml) [b]						
			T	U	V	X	Y×5	Z	R
1	1	Metabolic inhibition	3.0	2.1	1.7	24.6	23.0	<0.1	ND
		Cytopathic	4.8	2.0	4.0	26.9	14.0	<0.1	ND
	2	Cytopathic	5.6	4.0	3.6	35.5	39.5	<0.1	0.4
	3	Cytopathic	7.1	2.7	2.1	31.0	27.5	≤0.7	3.0
2	1	Metabolic inhibition	4.3	3.9	1.5	14.5	19.5	<0.0	ND
		Cytopathic	10.5	3.7	1.7	28.8	34.0	<0.2	ND
	2	Cytopathic	8.0	6.3	1.1	31.8	56.5	0.2	0.9
	3	Cytopathic	9.1	3.8	≤1.4	30.0	29.5	≤0.8	5.9
3	1	Metabolic inhibition	6.5	3.3	1.1	61.7	45.9	<0.0	ND
		Cytopathic	17.4	7.2	1.3	64.6	93.0	<0.2	ND
	2	Cytopathic	12.5	5.0	0.7	50.2	70.5	<0.1	0.3
	3	Cytopathic	12.0	7.1	≤1.7	67.7	66.5	≤0.7	2.4

[a] Standard preparations were assigned a potency of 10 units per ampoule.
[b] ND = not done.

TABLE 4
MEAN POTENCIES OF THE INTERNATIONAL REFERENCE PREPARATIONS RELATIVE TO THE PROPOSED INTERNATIONAL STANDARDS (10 units/ml) [a]

Test	Type 1		Type 2		Type 3	
	Number of assays	Relative potency (units/ml)	Number of assays	Relative potency (units/ml)	Number of assays	Relative potency (units/ml)
Cytopathic	6	3.9	4	23.2	4	4.3
Metabolic inhibition	8	5.2	10	12.3	8	5.0
Over-all mean	14	4.6	14	14.8	12	4.8

[a] Estimated at the National Institute for Medical Research, London.

TABLE 5
VARIANCES (LOGARITHMIC)

Laboratory	Type	s_R^2	d.f.	s_T^2	d.f.	s_R^2 (mean)	d.f.
1 (cytopathic)	1	0.0113	15	0.1905 [a]	3		
	2	0.0107	15	0.1661 [a]	3	0.0189	45
	3	0.0347	15	0.0244	3		
1 (metabolic inhibition)	1	0.0133	15	0.0357	3		
	2	0.0104	15	0.0682 [b]	3	0.0107	45
	3	0.0084	15	0.0132	3		
2	1	0.0214	12	0.0139	2		
	2	0.0451	12	0.1304	2	0.0296	34
	3	0.0208	10	0.0397	2		
3	1	0.0204	18	0.0031	3		
	2	0.0166	15	0.0651 [c]	3	0.0212	45
	3	0.0319	12	0.0075	3		

[a] Significant at P = 0.001. [b] Significant at P = 0.01. [c] Significant at P = 0.05.

were established as British standards (Perkins & Evans, 1959). Table 7 and the following statement are reproduced from their paper:

"These results indicate that there was little variation in antibody titre from ampoule to ampoule with each of the three standard preparations. The results also give an indication of the degree of stability of both the dry and reconstituted standards."

The weight of total solids per ampoule was also determined by them for each of the three standard preparations, and their results are reproduced in Table 8.

No further tests were carried out.

Specificity

Besides the assays described, laboratory 3 also examined the sera for cross-reactions between types.

TABLE 6
STANDARD ERRORS (SE) AND 95% LIMITS OF ERRORS

Laboratory	Single titrations		Average of n titrations				
			n	For titres		For potencies	
	SE (log scale)	Limits (% of titre)		SE (log scale)	Limits (% of titre)	SE (log scale)	Limits (% of potency)
1	0.122	56-179	4	0.061	75-133	0.086	67-149
2	0.172	45-221	3	0.098	64-157	0.138	53-189
3	0.146	50-200	4	0.073	71-140	0.103	62-161

TABLE 7

POLIOMYELITIS ANTIBODY TITRES OF PROPOSED STANDARD ANTISERA OBTAINED BY THE CYTOPATHIC TEST [a]

Test	Neutralizing antibody titres of Standard Sera		
	Type 1	Type 2	Type 3
Sera from different ampoules titrated in different tests immediately after reconstitution	960	960	960
	1 280	1 280	960
	1 280	1 280	1 280
	1 920	960	1 280
	1 280	640	1 280
	1 280	480	1 280
Reconstituted sera titrated:			
Immediately after reconstitution	1 280	640	1 280
After storage at 4°C for 4 months	1 280	960	960
After storage at 4°C for 5 months	1 280	960	960
After incubation at 37°C for 21 days	800	640	800
Sera titrated:			
From refrigerated ampoules	960	960	960
From 2 ampoules heated at 37°C for 40 days	1 120 960	320 480	800 640
From 2 ampoules heated at 56°C for 20 days	480 480	240 320	400 320
From ampoules heated at 100°C for 5 minutes	640	640	480
From ampoules heated at 100°C for 15 minutes	480	480	480

[a] Reproduced, by permission, from Perkins & Evans (1959).

It was found that the proposed standard sera as well as the International Reference Preparations behaved as monospecific when tested in dilutions of 1 : 16 and 1 : 8, whereas all other sera showed cross-reactions to the other types. The monospecificity of serum X could not be confirmed, as cross-reactions were found in the 1 : 16 dilution.

USE OF THE INTERNATIONAL STANDARDS FOR ANTI-POLIOVIRUS SERA

Since the stock of these Standards is limited, their use in daily routine all over the world is not possible. It will be necessary to establish national or laboratory substandards for such use. These substandards should be calibrated against the International Standards at reasonable intervals. Any anti-poliovirus sera with reasonably high titres could be used as substandards. For calibration of substandards any method could, in principle, be used, but the method in common use in the laboratory should be given preference. Care must be taken if methods are used by which the International Standards have not been tested previously. If an ampoule of the International Standard is opened and the contents completely dissolved in 1 ml redistilled water, this solution can be considered for all practical purposes to have a concentration of 10 international units per millilitre and in neutralization tests similar to those used in this collaborative study will probably have titres between 100 and 500. It is essential that preparations to be compared should be titrated

TABLE 8
WEIGHT OF TOTAL SOLIDS PER AMPOULE OF THE PROPOSED STANDARD POLIOMYELITIS ANTISERA [a]

Virus type	Weight of total solids in each of nine ampoules of each of three Standard Antisera (g)								
1	0.1084	0.1075	0.1076	0.1081	0.1079	0.1076	0.1087	0.1070	0.1074
2	0.1062	0.1057	0.1043	0.1040	0.1047	0.1040	0.1042	0.1040	0.1039
3	0.1054	0.1048	0.1045	0.1047	0.1058	0.1036	0.1062	0.1052	0.1026

	Mean weight (g)	Standard deviation (σ)	Coefficient of variation (%)
1	0.1078	0.00053	0.491
2	0.1046	0.00084	0.798
3	0.1048	0.00111	1.058

[a] Reproduced, by permission, from Perkins & Evans (1959).

under identical conditions and on the same day. In some instances the simple estimations used in this report may not suffice, and more elaborate calculations may have to be used. Although sera under test may have been compared to the calibrated substandard, their potency should be expressed in international units.

CONCLUSIONS

A collaborative study in four laboratories showed that the proposed standard anti-poliovirus sera types 1, 2, and 3 are suitable for establishment as International Standards in spite of the fact that there was some degree of heterogeneity between methods.

In accordance with the British units for poliomyelitis antisera Types 1, 2 and 3, and with the acceptance of collaborating laboratories, the three sera are established as International Standards for Anti-Poliovirus Sera, Types 1, 2 and 3, with units as given below:

For the Type 1 preparation: 1 unit is 10.78 mg
For the Type 2 preparation: 1 unit is 10.46 mg
For the Type 3 preparation: 1 unit is 10.48 mg

For practical purposes it will serve to consider the contents of one ampoule as equal to 10 international units.

RÉSUMÉ

Une étude poursuivie en collaboration dans quatre laboratoires de quatre pays a montré que les sérums antipoliomyélitiques de types 1, 2 et 3 préparés par le National Institute for Medical Research de Londres pouvaient être adoptés comme Etalons internationaux, en dépit d'un certain degré d'hétérogénéité des méthodes utilisées.

D'accord avec le système britannique d'unités pour les sérums antipoliomyélitiques de types 1, 2 et 3 et après agrément des laboratoires ayant collaboré à cette étude, les trois sérums sont établis comme Etalons internationaux de sérums antipoliomyélitiques, types 1, 2 et 3 avec les unités suivantes:

Pour le sérum de type 1: 1 unité correspond à 10,78 mg
Pour le sérum de type 2: 1 unité correspond à 10,46 mg
Pour le sérum de type 3: 1 unité correspond à 10,48 mg

En pratique, l'on doit considérer qu'une ampoule contient 10 unités internationales.

Ces Etalons remplacent les Préparations internationales de Référence en usage depuis 1958.

REFERENCES

Bentzon, M. W., Evans, D. G., Maaløe, O., Perkins, F. T. & Spaun, J. (1962) *Bull. Wld Hlth Org.*, **26**, 341

Gard, S., Weasler, R., Fagraeus, A., Svedmyr, A. & Olin, G. (1956) *Arch. Virusforsch.*, **6**, 401

Perkins, F. T. & Evans, D. G. (1959) *Brit. med. J.*, **1**, 1549

Salk, J. E., Younger, J. S. & Ward, E. N. (1954) *Amer. J. Hyg.*, **60**, 214

Spaun, J. (1956) *Acta path. microbiol. scand.*, **39**, 469

WHO Expert Committee on Biological Standardization (1959) *Wld Hlth Org. techn. Rep. Ser.*, **172**, 15

WHO Expert Committee on Biological Standardization (1961) *Wld Hlth Org. techn. Rep. Ser.*, **222**, 19